Marine bioprospecting - searching for interesting and unique genes, biomolecules and organisms in the marine environment

BIOPROSP 2004

Symposium in marine bioprospecting

Tromsø Science Park Conference Centre
Tromsø, Norway, October 13th – 14th

Objectives of the symposium:

- Visualize activities and commercial opportunities in marine bioprospecting.
- Be a national meeting place for participants and collaborating partners within marine bioprospecting.

Organizers:
The MABIT program (Marine Biotechnology program in Tromsø)
Bioklynge Nord, Innovation Norway, Tromsø
The Research Council of Norway

Program committee:
Guri Eggset, Margrethe Esaiassen, Dag Rune Gjellesvik, Karl-Johan Jakola, Trond Jørgensen, Kristin Rasmussen Modalsli, Zølvi Pedersen, Erling Sandsdalen, Even Stenberg, Klara Stensvåg, Olaf Styrvold, Nils P. Willassen

Welcome to BIOPROSP 2004!
After the first assembling on the subject marine bioprospecting in Tromsø in 2002, we want to follow up with a new symposium. During the last years, efforts have been directed towards building activities and competence within marine bioprospecting in Norway. Hopefully, this time a broad range of activities will be presented. Speakers have also been invited from several countries. We hope that the objectives of the symposium and your own expectations will be fulfilled, and are looking forward to an interesting meeting and enjoyable stay for all the participants!

The organizers

MABIT
Marin bioteknologi i Tromsø

Norges forskningsråd

INNOVASJON NORGE

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1640-1700 By-products of cultured blue mussel: BioGlue (working title). (28)
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Sigve Nordrum, Norwegian Ministry of Fisheries.

0930-1015 Bioprospecting a sustainable industry for Norway?
Kristin Modalsli, The Research Council of Norway.

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Thor Amlie, Norwegian Bioindustry Association

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1340-1400 From science to administration.
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Anders Goksøyr, Biosense Laboratories AS

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Jan Raa, Biotec Pharmacon ASA.

List of Posters

Abstracts ~ oral presentations

Biocatalytic potential of enzymes from extremophiles

Michael J. Danson

Centre for Extremophile Research, Department of Biology and Biochemistry

University of Bath, Bath, BA2 7AY, UK.

Extremophiles are organisms that require, and grow optimally in, earth's extreme environments of temperature (-2 to 10°C and 60 to 115°C), salinity (3-5 M NaCl), pH (pH 0-4 and 9-12), and/or pressure. Consequently, their cellular components are naturally adapted to survive and function in these extremes, including their enzymes that are therefore known as *extremozymes*. Many extremophiles, particularly the hyperthermophiles, the extreme halophiles and the methanogens, belong to the Domain Archaea, which is an evolutionary lineage that is distinct from the Bacteria and Eukarya. Archaea have a number of unusual metabolic pathways and consequently possess novel enzymes with modified structures and substrate specificities.

Therefore, Archaea are a potential source of hyperstable enzymes with novel catalytic activities

This seminar will explore the nature and potential of extremozymes, and will consider:

- **The structural basis of enzyme thermostability and cold activity**

  Through a comparison of high-resolution structures of a single enzyme from organisms spanning the biological range of temperatures at which life exists, trends in the structures have been correlated with their increasing thermostability. Data from other extremozymes will be combined with mutational studies to identify common features, and the importance of inter-subunit interactions in oligomeric enzymes will be stressed.
- **The relationship between catalytic activity and stability**

  Thermo*stability* is necessary for, but does not guarantee, thermo*activity*. A new concept of true temperature optima that explores the relationship between stability and activity will be presented and described mathematically. The importance of this idea to engineering enzymes to modify their thermal properties, by both rational design and by directed enzyme evolution, will be stressed.
- **Catalytic novelty and promiscuity** A thermostable aldolase with unusual substrate specificity and steroechemistry will be described to illustrate the potential novelty of enzymes from the Archaea, and to show how both substrate engineering and protein mutagenesis can be used to manipulate catalytic and mechanistic promiscuity to suit biocatalytic needs.

[1] Danson, M.J. & Hough, M.J. (2003) *Encyclopaedia of Life Support Systems (http://www.eolss.net. Knowledge Foundations Area: Extremophiles).* pp.1-28. **Thermostability and thermoactivity of extremozymes.**

[2] Daniel, R.M., Danson, M.J. & Eisenthal, R. (2001) *Trends in Biochem.* 26, 223-225. **The temperature optima of enzymes: a new perspective on an old phenomenon.**

[3] Peterson, M., Eisenthal, R., Danson, M.J., Spence, A. & Daniel, R.M. (2004) *J. Biol. Chem.* 279, 20717-20722. **A new intrinsic thermal parameter for enzymes reveals true temperature optima.**

[4] Lamble, H.J., Heyer, N.I., Bull, S.D., Hough, D.W. & Danson, M.J. (2003) *J. Biol. Chem.* 278, 34066-34072. **Metabolic pathway promiscuity in the Archaeon *Sulfolobus solfataricus* revealed by studies on glucose dehydrogenase and 2-keto-3-deoxygluconate aldolase.**

[5] Theodossis, A., Walden, H., Westwick, E.J., Connaris, H., Lamble, H.J., Hough, D.W., Danson, M.J. & Taylor, G.L. (2004) *J. Biol. Chem.* In Press. **The structural basis for substrate promiscuity in a hyperthermophilic aldolase.**

NATURAL MEDICINES FROM THE SEA

By Bulent Kukurtcu, D

Head of Biodiversity and Expeditions Department, R&D, PharmaMar, S.A.U.

Nature has been the source of the chemical skeletons of a very large fraction of the active molecules in today's key drugs for treatment of human disease. The oceans make up 70% of our planet's surface, and their biodiversity and evolutionary processes make them a rich source of new chemical entities, yet they have been relatively unexploited. We know that marine organisms evolved for millions of years before terrestrial organisms existed and have developed molecules with biological activity in order to survive in a hostile environment. PharmaMar, a biopharmaceutical company dedicated to the development of marine natural products for oncology, believes that marine-derived molecules hold great potential for use in human medicine~~s~~.

To this end the company, together with academic collaborators, have been collecting marine specimens for nearly two decades building a very rich library unique in the industry. Screening of this library through a variety of assays, isolation and elucidation of the structures of the active molecules has confirmed the potential of marine biodiversity in supplying a panoply of novel chemical entities for drug development. The molecules identified span a broad spectrum of structures from terpenes thru tetrahydro isoquinolines to cyclic depsipeptides. The development of scalable synthetic production processes for those presenting appropriate therapeutic windows in preclinical models has transformed them into authentic drug candidates.

PharmaMar's successful efforts have resulted in an extensive portfolio of new promising anti-cancer compounds and we currently have 4 anticancer agents in clinical trials, Yondelis^{TM} (ET-743), Aplidin^{R}, Kahalalide F and ES-285. These compounds, all marine-derived, have novel chemical structures and novel mechanisms of action.

In addition, fifteen marine-derived compounds are already in preclinical development. Of the other molecules already screened, more than a hundred have shown early potential. Research and collaborations with scientific institutions and universities are instrumental for our R & D strategy in order to further study and develop such a large number of interesting candidates.

APOPTOSIS-INDUCING ANTI-CANCER DRUGS

Stein Ove Døskeland

Institute of Biomedicin, University of Bergen.

The drug industry is struggling to find novel drug candidates for the treatment of important and common diseases, including cancer. The use of "brute force" chemical synthesis of novel compounds and complex high-throughput screening has met with less success than expected. This is in part due to too few well-defined, easily screenable drug targets in "Big Pharma", and the redundancy of cell signaling, which make rational drug design more challenging than hitherto expected. There is therefore a renaissance of the "old-fashioned" bioprospecting approach, which relies on "pre-screening" by nature itself. This renaissance is helped by advances in large scale culturing of marine micro-organisms, in chemical purification and in structure elucidation of natural compounds. Today, only a small amount of an interesting ("lead") compound can suffice as basis for further drug design, using advanced computer-assisted modeling and hemisynthesis. When coupled to progress in fundamental cell biology, the bioprospecting of the under-exploited marine micro-organisms promises to be an efficient pathway towards novel drug candidates.

We have found that several compounds from marine micro-organisms can induce unusual types of apoptotic death in mammalian cells, including cells from patients with myeloid leukemia. Examples of how such compounds can kill cancer cells and how they can be analyzed for efficacy and toxicity in experimental rodent models of leukemia will be presented.

Antibacterial peptides from invertebrates

Tor Haug

Department of Marine Biotechnology, Norwegian College of Fishery Science, University of Tromsø, Norway

Gene-encoded, ribosomally synthesized antimicrobial peptides (AMPs) are widespread in nature, and have been identified in various species, including bacteria, fungi, plants, invertebrates and vertebrates. In eukaryotes they form the first line of host defence against pathogenic infections and are a key component of the innate immune system in invertebrates and vertebrates. It is generally accepted that most of these peptides interact with bacterial membranes although different mechanisms may be used by different peptides under different conditions for killing. The peptides have an enormous variety of sequences and structures, but certain features are common. Most naturally occurring AMPs carry a net positive charge (i.e. cationic) and are composed of 12-100 residues where approximately 50% of them are hydrophobic. Many of these peptides are endowed with several favourable properties:

1) Activity against a broad spectrum of microbes, which include bacteria, fungi, protozoa and enveloped viruses.
2) Potent activity against pathogens, which are resistant to conventional antibiotics.
3) Rapid lytic activity.
4) Rarely induce bacterial resistance.
5) Low toxicity for eukaryotic cells.
6) Mechanism(s) of action dissimilar to conventional antibiotics.
7) Additional immunomodulatory properties.

Due to these properties, AMPs have emerged as one of the most promising candidates for a new class of antibiotics. Invertebrate AMPs are mainly produced by phagocytic blood cells and epithelial cells. AMPs have in recent years been isolated from various marine invertebrates, including crustaceans, molluscs, tunicates, nematodes, annelids, and horseshoe crabs. We have (at the Dept. of Marine Biotechnology) recently isolated and characterized multiple novel antibacterial peptides from the blood cells of a number of marine invertebrates, including crustaceans, molluscs, echinoderms and cnidarians. Some of these peptides display potent activity against both Gram-positive and Gram-negative bacteria.

Mining for antibiotic-producing bacteria in the Trondheim fjord

[1]Bredholt H., [1]Fjærvik E., [1]Hakvåg S., [2]Josefsen K.D., [2]Sletta H., [3]Johnsen G., [2]Ellingsen T.E., [1]Zotchev S.B.

[1]Department of Biotechnology, NTNU, Trondheim; [2]SINTEF Materials and Chemistry, Trondheim; [3]Department of Biology, NTNU, Trondheim.

The discovery and use of antibiotics over the last 60 years have drastically reduced the mortality caused by infectious diseases. However, over the past two decades many bacterial and fungal pathogens have developed resistance to most, if not all, medically important antibiotics. This trend prompted the new search for anti-infective agents with novel mechanisms of action. The new antibiotics have traditionally been isolated from natural sources, exploiting the antagonistic relationship in different ecological niches. From this point of view, bacteria belonging to the family *Actinomycetaceae* represent a rich source for new antibiotics, since they have been shown to produce a wide range of biologically active secondary metabolites.

We have decided to focus on isolation of potential antibiotic producers from the marine environment in the Trondheimsfjord, since it has been demonstrated that some actinomycetes isolated from the sea produce novel and structurally unusual antibiotics.

We are pursuing two parallel strategies, isolating actinomycete bacteria from marine sediments and the upper layer of a water surface (neuston layer). Sediments are sampled by either divers or, for deep-water sediments, by a box corer, and pre-treated before plating on different isolation agar media to facilitate isolation of actinomycetes. The neuston layer is sampled using teflon plates, and cell suspensions are plated on the isolation agar media without pre-treatment. So far, over 3500 actinomycete colonies have been isolated, and the methods for efficient screening of their biological activities using the robot lab are being established. Preliminary screening utilising Gram-positive and Gram-negative bacteria as well as non-filamentous fungi as test organisms have shown that over 70 % of our actinomycete isolates exhibit antagonistic activity. We are also working on the development of methods for rapid identification of actinomycetes using PCR techniques that should enable us to assemble an annotated library of antibiotic-producing actinomycetes isolated from the Trondheimsfjord. The strategies for isolation and identification of novel antibiotics using recently installed LC-MS/MS and TOF equipment are under development.

THE USE OF DESIGNED BIOCATALYSTS FOR EXPLOITATION OF CHITIN AND CHITOSAN

Vincent G.H. Eijsink

Agricultural University of Norway

Chitin is produced by a variety of organisms, such as crustaceans, molluscs, algae, insects, fungi and yeasts. Each year about 30 million tons of shellfish are harvested worldwide, and shellfish waste is currently the major source of chitin available to the industry. Chitin is an insoluble polysaccharide, composed of β1,4- linked *N*-acetyl-D-glucosamine (GlcNAc) residues. The name chitosan refers collectively to water-soluble copolymers of GlcNAc and D-glucosamine (GlcN) obtained by partial *N*-deacetylation of chitin. Currently only a fraction of chitin-containing biomass is exploited, mainly in the form of chitosan.

There is commercial interest in the conversion of chitin or chitosan to bioactive chito-oligosaccharides (CHOS) with specific lengths and acetylation patterns, since such CHOS may find applications as agrochemical, pharmaceutical or neutraceutical. Chemical production of CHOS with specific lengths and acetylation patterns is difficult (as chemical oligosaccharide engineering in general). Therefore, enzymes need to be used to convert chitin or chitosan to CHOS and to enrich CHOS mixtures for bioactive components. Chitinases and chitosanases may be used to convert the polymers to shorter fragments, including "building blocks" for further biocatalytic or chemical modifications. Further biocatalytic modifications may include specific deacetylations with deacetylases and the use of so-called "glycosynthase technology", where specially designed variants of hydrolytic enzymes (chitinases) are use to couple short oligosaccharide building blocks together.

Together with chitosan experts at The Norwegian Biopolymer Laboratory (NOBIPOL), we study enzymes for the conversion of chitin and chitosan. Several relevant enzymes have been overexpressed ("genome mining") and characterized in detail. Enzyme engineering technology is used to tailor the substrate and product specificities of these enzymes and to improve industrially important properties such as stability at high temperature and extreme pH. Promising enzymes are used to convert various types of well-characterized chitosans. The resulting oligosaccharides are tested for bioactivity and used as input for further compound development. One goal is to find optimal combinations of certain types of chitosans and certain enzyme variants. Thus, we aim at creating knowledge-intensive technology to add value to an important marine resource. The current status of this work will be reported.

Some recent publications:

- Structural insights into the catalytic mechanism of a family 18 exochitinase; D.M.F. Van Aalten, D. Komander, B. Synstad, S. Gåseidnes, M.G. Peter, V.G.H. Eijsink; *Proc. Natl. Acad. Sci. USA* **98** (2001) 8979-8984
- High resolution structures of a chitinase complexed with natural product cyclopentapeptide inhibitors – mimicry of carbohydrate substrate; D. Houston, K. Shiomi, N. Arai, S. Omura, M.G. Peter, A. Turberg, B. Synstad, V.G.H. Eijsink, D.M.F.van Aalten; *Proc. Natl. Acad. Sci. USA* **99** (2002) 9127-9132.
- Stabilization of a chitinase from *Serratia marcescens* by Gly -> Ala and Xxx -> Pro mutations; S. Gåseidnes, B. Synstad, X. Jia, H. Kjellesvik, G. Vriend, V.G.H. Eijsink; *Protein Engineering* **16** (2003) 841-846.
- Interactions of a family 18 chitinase with the designed inhibitor HM508 and its degradation product, chitobiono-delta-lactone; G. Vaaje-Kolstad, A. Vasella, M.G. Peter, C. Netter, D.R. Houston, B. Westereng, B. Synstad, V.G.H. Eijsink, D.M.F. van Aalten; *J. Biol. Chem.* **279** (2004) 3612-3619.
- Mutational and computational analysis of the role of conserved residues in the active site of a family 18 chitinase; B. Synstad, S. Gåseidnes, D.M.F. van Aalten, G. Vriend, J.E.Nielsen, V.G.H. Eijsink; *Eur. J. Biochem.* **271** (2004) 253-262.
- Degradation of chitosans with Chitinase B from *Serratia marcescens*; production of chito-oligosaccharides and insight in enzyme processivity; A. Sørbotten, S.J. Horn, V.G.H. Eijsink, K.M. Vårum, submitted for publication.

Better photosynthesis - an arctic search for the world's best RuBisCOs

Edward Hough

NORSTRUCT/Inst. of Chemistry, University of Tromsø

The first step in the assimilation of atmospheric CO2 into the biosphere is catalysed by the enzyme ribulose-1,5-bisphosphate carboxylase/oxidase (RuBisCO). As its name implies RuBisCO actually catalyses two competing reactions, incorporation of CO2 into Ribulose-1,5- bisphosphate (RuBP) to generate two molecules of 3-phosphoglycerate (photosynthesis) or oxidation of RuBP ultimately to CO2 and 3-phosphoglycerate and Glyoxalate (photorespiration). These reactions occur in the same active site; in essence RuBisCO is unable to distinguish between CO2 and O2 and is thus highly inefficient.

The whole basis for food supply on earth lies in the fact that the balance (specificity factor) between the competing reactions is slightly in favour of the photosynthetic reaction. Nature compensates for this by producing RuBisCO in enormous quantities.

RuBisCOs with the most favourable specificity factors are found in marine alga.

The EU-project which will be presented involves harvesting, characterisation, and structural and biochemical analysis of algae from arctic waters in the search for the worldâ€™s best RuBisCOs. The ultimate aim is the incorporation of genes for these into higher plants to improve agricultural yields.

Screening for cold adapted enzymes

Elin Moe[a], Ingar Leiros[b], Arne Smalås[a] and Nils Peder Willassen[c]

[a]The Norwegian Structural Biology Centre, University of Tromsø, [b]The European Synchrotron Radiation Facility, 38043 Grenoble Cedex, France. [c]Department of Molecular Biotechnology, Institute of Medical Biology, Faculty of Medicine

Cold adapted enzymes are characterised by increased catalytic efficiency and decreased temperature stability compared to their mesophilic and thermophilic counterparts. The reduced stability is probably caused by a more flexible structure, and is explained by the need for rapid conformational changes during catalysis at low temperatures. Amino acid substitutions that alter hydrophobic and intramolecular interactions of the enzymes are believed to be the source of the increased flexibility. In the present study structural analysis and characterisation of mutants of recombinant cod uracil-DNA N-glycosylase (rcUNG) are performed in order to obtain more information about the molecular basis for cold adaptation of this enzyme. The results suggest that cod UNG has developed an increased structural flexibility by reducing the number of ion pairs near the substrate-binding site in order to perform necessary conformational changes at low temperatures. In addition, the enzyme seems to have optimised the electrostatic surface potential near the catalytic site in order to improve the interactions with the substrate.

"The bottom is the limit"

Inge W. Nilsen, Fiskeriforskning

Cold-adapted enzymes include the display of cold-activity and/or thermolability, or at least molecules of higher efficiency in their intermolecular interactions. These features may be subtly expressed *in vivo* but still provide the necessary adaptation to temperature for the host to succeed in its environment.
The presentation will give an overview of the current status of our work, and plans for the future, on enzymes, their genes as well as enzyme-modulators from cold-adapted marine organisms. A need to ”know-your-sample” will be emphasised by examples. Some issues of funding and commercial interaction will also be considered.

The contribution of camparative genomics to our understanding of bacterial pathogenesis

Nicholas Thomson

Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK

Bacterial whole genome sequencing has given us an unprecedented insight into the genomic architecture and evolution of many important human, plant and animal pathogens. It has highlighted the contribution of the core and
accessory regions within a genome to overall genetic diversity and their influence on the pathogenic potential of the bacterium in question. The accessory genome shows great variety in its make up, consisting of large integrative elements such as prophage, pathogenicity islands, plasmids and transposable elements. Our understanding of the genomics of these pathogens will greatly aid our ability to understand how these microbes cause disease, with the aim of designing new strategies for their control.

High Throughput Sample Preparation and Screening of Marine Microorganisms for Drug Discovery

Dr. Trevor P. Castor, Aphios Corporation, 3-E Gill Street, Woburn, MA 01801, USA

High throughput screening of partially purified marine microorganism fractions is being conducted to enhance the discovery of novel marine therapeutics for infectious diseases, cancer and other human diseases. To conduct this screening, we have developed a rapid sample preparation method that has been automated. This method utilizes *SuperFluids™* (supercritical, critical or near-critical fluids with or without polar cosolvents such as an alcohol) for first, cell disruption, and second, polarity-guided fractional extraction of bioactive constituents. Aphios has established a library of more than 1,400 unique marine microorganisms from diverse environments, including deep-sea sediments to shallow water mangrove swamps, tropical waters to temperate oceans, hydrothermal vents as well as normal saline to hypersaline conditions. The microorganisms are fermented in four different marine media designed to maximize the diversity of secondary metabolites. Biodiversity is further enhanced by utilizing *SuperFluids™* to produce partially purified fractions of increasing polarity. In a direct, large-scale and broad comparison (332,800 tests, 8,320 fractions in 40 different screens) with conventional organic phase (butanol) extraction by the Bristol-Myers Squibb Company, we have demonstrated that this process will increase the recovery and diversity of secondary metabolites, reduce interference from nuisance compounds and minimize background noise in sensitive enzymatic and molecular-based screens. Our pharmaceutical screening experiences have indicated a much higher proportion of bioactive "hits" in certain screens from these *SuperFluids™* fractions, at lower than normal metabolite concentrations. Aphios is utilizing this process of high throughput sample preparation and screening to develop novel anti-infective therapeutics for Gingivitis, Multi-Drug Resistant (MDR) Bacteria, Influenza, HIV and Smallpox.

Bioprospecting for microorganisms in the sea surface microlayer

[1]Josefsen, K.D., [2]Hakvåg, S., [2]Aakvik, T., [1]Degnes, K.F., [1]Bruheim, P., [1]Øino, N., [1]Klinkenberg, G., [1]Sletta, H., [2]Valla, S., [2]Zotchev, S.B., [2]Strøm, A., [2]E. Fjærvik, [1]Ellingsen, T.E.

[1]SINTEF Materials and Chemistry, Trondheim; [2]Department of Biotechnology, NTNU, Trondheim.

The overall aim of this recently started project is to isolate microorganisms from the sea surface microlayer that produce valuable products and have an industrial potential. We want to apply our bioprospecting platform using a multiple screening strategy based on an automated system, and search for producers of several types of products simultaneously. We have chosen to focus on carotenoids, antibiotics, and polyunsaturated fatty acids, all of commercial value. However, the culture collections and libraries generated in this project will be a good starting point also for later bioprospecting for other products.

The aquatic surface below the air/water interface have been divided into a series of sublayers; the thin surface nanolayer (<~1 µm) enriched in surface-active compounds; the surface microlayer (<~1000 µm) with high densities of particles and microorganisms; and the surface millilayer (<~10 mm) inhabited primarily by small animals, and the eggs and larvae of fish and invertebrates. The reported enrichment of viable bacteria per unit volume in the surface microlayer relative to the underlying water is often 10-100 times. Total count studies have yielded enrichment factors in the same range. There is evidence that the microbial community of the surface microlayer differs from that of the underlying water.

Two approaches are employed in the project: direct isolation of growing (colony forming) microorganisms, and construction of a metagenome library from the unculturable fraction (90-99 %) of the flora. Samples of the surface microlayer for direct isolation of colony forming microorganisms have mostly been collected with Teflon plates. For the larger volumes required for the gene library a screen sampler is employed. Results and experiences from the first phase of the project will be presented.

Potentially carotenoid producing microorganisms, as judged by their colony color, are common in the surface microlayer and constitute up to one third of the colony forming units. Several hundred isolates have been obtained. LC-MS analyses show that different carotenoids dominate in different isolates.

The thraustochytrids are a group of eukaryotic microorganism that are placed in a unique phylum, *Laborynthulomycota*. Some species, particularly within the genera *Thraustochytrium* and *Schizochytrium*, are known to accumulate large amount fat (up to 50 % of dry body weight) and DHA accounts for up to 35 % of their fatty acids. Around 20 assumed thraustochytrids have so far been isolated from the surface microlayer, but it remains to be determined to what degree they accumulate fat and DHA.

The bioprospecting for antibiotic-producing microorganisms from the surface microlayer has concentrated on actinomycetes, and has so far yielded around 300 strains, of which 30-50 %, depending on the test organism, show antimicrobial activity.

Drugs from the sea

Marbank and Marbio, a repository of marine organisms and a high-throughput screening platform

Kjersti Lie Gabrielsen and Jeanette Hammer Andersen

University of Tromsø

A repository of marine organisms and a high-throughput screening platform, Marbank and Marbio, are being established in Tromsø. Marbank will have a national responsibility for collection and preservation of marine resources/ organisms for research, commercial and exploitation purposes. The material to be archived and stored in the Marbank repository will include genetic and biological material from marine microorganisms, plankton, algae, invertebrates and vertebrates. The aim of Marbio is bioprospecting a large number of marine organisms from Arctic and sub-Arctic regions for potential drugs and/or lead compounds. Together with Marbank, the platform has the capacity to collect, extract and purify molecules with unique bioactivities within the most important drug areas, including molecules with anti-bacterial, antiviral, anticancer and immunostimulatory or anti-inflammatory action, in addition to various enzyme activities.

Marine bacteria; diversity and trends in survey methods

Bjarne Landfald

Norwegian College of Fishery Science, University of Tromsø, 9037 Tromsø

The search for novel, biotechnologically interesting traits among marine bacteria has been strongly restricted by "the great plate count anomaly", i.e., the fact that just a small fraction of the various bacterial types that are present, are culturable by established microbiological methods. This challenge is presently met by two strategies: Through "metagenomics", total microbial DNA from selected environments are extracted, shotgun cloned and sequenced to obtain more or less complete coverage of the genetic pool of the environment. The other strategy is the development of highly refined cultivation techniques to obtain pure cultures from phylogenetic groups that have hitherto been considered "unculturable." Both approaches are presently giving fascinating new insights into the physiological and biochemical repertoire of marine bacteria.

Natural gas and biotechnology: Bioprospecting of methane-oxidizing bacteria

Nils-Kåre Birkeland

Department of Biology, University of Bergen, Box 7800, N-5020, Bergen

Methanotrophic bacteria are widespread in nature and can grow on methane, the major component of natural gas, as the sole source of carbon and energy. As one of the world's major producers of natural gas, Norway has a great potential for developing an extensive industry based on the use of natural gas in large-scale production of bioprotein and biochemicals by methanotrophs. A large-scale unit for production of single-cell protein is already established, and the bioprotein has been licensed as feed for fish and animals. We have initiated a screening for methane-oxidizing bacteria in a variety of methane-containing environments such as methane seeps on the ocean floor, hot springs, gas-fields in the North Sea, landfills and tropical rice-fields. The aim is to provide strains with improved properties for fermentation of crude natural gas, genetic elements for development of tools for genetic manipulation, and strains that produce bioactive substances such as bacteriocins and antibiotics. Organisms are enriched and isolated under a wide range of cultural conditions and screened for a variety of biological properties. Analyses of the diversity of methanotrophs are also carried out using molecular ecology techniques.

SEAFOOD and marine ingredients in functional food

Professor Edel O. Elvevoll, Norwegian College of Fishery Science, Department of Marine Biotechnology, University of Tromsø, 9037 Tromsø, Norway

Scientific research constantly provides new insights in the interaction between genetic predisposition, specific health risks and nutritional needs, and the functioning of separate nutrients. The role of food as an agent for improving health has been proposed as a new class of food- functional foods.

Scientific data shows that the consumption of seafood and marine oil containing omega-3 polyunsaturated fatty acids (PUFAs) reduces the risk of coronary heart disease, decreases mild hypertension, prevents certain cardiac arrhythmia, and sudden death, lowers the incidence of diabetes, and appears to alleviate symptoms of rheumatoid arthritis. It appears that omega-3 PUFAs play a vital role in the development and function of the nervous system (brain), photoreception (vision), and the reproductive system.

Additional components in seafood may be of importance for development of life style diseases. Potent peptides with high anti hypertensive activities and petides, which may modulate neuropeptide levels, have been isolated from fish waste. Protease inhibitors of the serpin family, or serine protease inhibitors, are a family of glycoproteins that include members involved in the control of blood coagulation, fibrinolysis, complement activation and inflammation processes, are also found. Calcium and vitamin D are other candidates. Antioxidants (tocopherols, ubiquinone, selenium, taurine, fish protein) have attracted special attention due to their possible prevention of low-density lipoprotein (LDL) oxidation.

Every year 30 million tons of waste is dumped around the world, and Norway alone has been "wasting" 150,000 tons a year. Fish waste may be sources for of proteins of high biological value, unsaturated essential fatty acids, vitamins and antioxidants, minerals or trace metals and physiological beneficial amino acids and peptides.

Chitosans – from water purification to gene delivery

Kjell M. Vårum

Norwegian Biopolymer Laboratory (NOBIPOL), Dept. of Biotechnology, Norwegian University of Science and Technology, N-7491 Trondheim, NORWAY

Chitin is one of the most abundant organic substances on earth, probably second only to cellulose. Crustacean shells from marine sources are used as raw materials for production of chitin, which is one of the three main components of the shells. Chitin is a linear polysaccharide composed of (1-4) linked GlcNAc units (**A**-unit). A severe limitation in the application of chitin is its insolubility in most aqueous solvents. This limitation is not sheared by the chitin-derivative chitosan, where the **A**-units in chitin have been de-N-acetylated to varying extents (0 to 60 % **A**-units), thereby introducing positively charged glucosamine units (**D**-units) in the polymer. Chitosans can thus be regarded as an amphiphilic polymer composed of the charged **D**-units and the more hydrophobic and neutral **A**-units with different chemical, physical and biological properties (Vårum & Smidsrød, 2004), and in the following some examples will be presented and discussed.

When the pH of an acidic chitosan solution is increased, the chitosan may stay in solution or precipitate, depending on the fraction of acetylated units (F_A) and the molecular weight. The neutral-solubility of chitosans was found to increase with increasing F_A and decreasing molecular weight. The pK_a-value of **D**-units in chitosans of varying F_A have been determined using electrophoretic light scattering and proton nmr-spectroscopy, and pK_a–values of 6.5-6.6 have been determined. Initial lysozyme degradation rates of chitosans were found to increase proportionally to F_A in the fourth power, and the same strong increase in degradation rate with F_A was found in experiments with human serum, suggesting that chitosans in serum are mainly depolymerized by lysozyme. Chitosans may be utilized to flocculate bacteria, and chitosans with different F_A have been evaluated as flocculants of bacteria using suspensions of Escherichia coli as model organism. With increasing F_A from 0 to 0.6, a more than 10-fold increase in the flocculation efficiencies were observed. Chitosans have been shown to form complexes with DNA, and promising results have been achieved with chitosans as a non-viral gene delivery system. The transfection efficiencies of chitosans were found to vary significantly with chemical composition, and chitosans with low F_A were most effective as transfection agents in vitro. The molecular weight and the molecular weight distribution of highly deacetylated chitosans have been found to affect complex formation and transfection efficiencies both in vitro and in vivo.

K.M. Vårum & O. Smidsrød (2004) In: Polysaccharides: Structural Diversity and Functional Versatility (ed.: S. Dumitriu), Chapter 26. In press.

Detection of Single Nucleotide Polymorphisms (SNPs) from Atlantic Salmon Expressed Sequence Tags (ESTs)

Hayes, B. [1,2], Lærdahl, J. [3], Lien, S.[2] , Berg, P[2]., Davidson, W.[4], Koop, B.[5], Adzhubei, A.[3], Høyheim, B.[6,7]

[1]AKVAFORSK, P.O. 5010, 1432 Ås, Norway, [2]Centre for Integrative Genetics, Ås, Norway, [3]Biotechnology Centre of Oslo, University of Oslo, Norway,[4]Simon Fraser University, Burnaby, British Columbia VSA 1S6 Canada, [5]Centre for Biomedical Research, University of Victoria, Victoria, Britich Columbia V8W 3NS Canada, [6]Norwegian School of Veterinary Science, BasAM-Genetics, Oslo, Norway [7]National Hospital, Dept of Dermatology, Oslo, Norway

Single nucleotide polymorphism (SNP) markers are variations among animals in the genetic code at specific sites in the genome. If the SNPs are in coding or regulatory regions of the genomes, they can alter the level or function of expressed proteins. For an important aquaculture species such as Atlantic salmon, this information could be used for example to search for novel gene variants in genes with effects on disease traits, in order to inform drug development and targetting, or to search for protein variants with effects on flesh colour, an economically important trait.

Our goal was to find SNP markers in the Atlantic salmon genome. We took 100, 866 expressed sequence tags (ESTs) sequences from Norwegian and Canadian databases. These ESTs were fragments of DNA expressed in a variety of tissues. Using the phred and phrap programs, the sequences were assembled into 17,660 overlapping clusters, or 'contigs'. The sequence of seventy five percent of these contigs had high quality matches with sequences of genes in public databases.

By comparing sequences within a contig, we were able to detect a large number of base substitutions. The PolyBayes program was used to predict whether a base substitution in the aligned sequences was sequencing error, or a true SNP. We detected 2,507 base substitutions with very high probability of being a true SNP. A public database of the SNPs is being developed. Further investigation and modelling is required to determine if any of the discovered SNPs alter protein functions.

Great scallop myostatin – the role as a negative muscle growth regulator also in invertebrates?

Andersen, Ø[1], Pagander, H[1], Strand, Ø[2], Magnesen, T[3], Johnston, IA[4]

[1]AKVAFORSK, P.O.Box 5010, 1432 Ås-NLH

[2]Inst. Marine Research, P.O.Box 1870 Nordnes, N-5817 Bergen

[3]SCALPRO AS, 5337 Rong

[4]Gatty Marine Laboratory, University of St. Andrews, Scotland

Cultivation of scallop has received much attention due to the increasing demand for high quality seafood products at the European market. The highly favourable conditions for growing the great scallop, *Pecten maximus*, along the Norwegian coastline give a unique opportunity to become a leading nation for scallop production. This project is focusing on the environmental and genetic regulation of the larval growth and development of the large adductor muscle, which is the primary product sold. The muscle fibre anatomy and recruitment have been studied for the first time in molluscs. Water temperatures of 14°C or 18°C at early developmental stages were shown to influence larval growth and survival. Genes encoding contractile proteins and regulatory factors have been partially isolated, including the negative muscle growth regulator myostatin. Great scallop myostatin showed about 50 % sequence to fish and mammalian myostatin in the active C-terminal portion. Studies of the sub-cellular location and the potential effects of myostatin on the recruitment of muscle fibres are in progress. Future studies will utilise subtractive hybridisation to discover novel genes responsible for muscle fibre production.

An immunoactive chrysolaminaran from the marine diatom *Chaetoceros mülle*r**i; Structural characterization and use in first feeding experiments with Cod *(Gadus morhua* L)**

Trond R. Størseth [1,2], Karina Hansen [1], Alexander H. Hansen [1], Kjell I. Reitan [1] and Jorunn Skjermo [1]

1) SINTEF Fisheries and Aquaculture, N-7465 Trondheim, Norway
2) 2) Norwegian University of Science and Technology, Dept. of Chemistry, N-7491 Trondheim, Norway

One class of immunostimulants are the â -(1 Æ3,1 Æ6)-glucans[1, 2]. Both high and low molecular weight compounds of this class have been shown to be immunostimulants[3]. The storage polysaccharides of diatoms are chrysolaminarans which are â -glucose polymers with branches in the â -(1 Æ2) and â -(1 Æ6) positions[4]. As the chrysolaminarans possess these structural features marine microalgae would be interesting as sources for immunostimulants. As part of the Norwegian Research Council (NFR) funded project MARBIM (NFR project 143450/140 – 01.10.2000-31.12.2004), SINTEF Fisheries and Aquaculture has been screening marine microalgae for potential immunostimulants. This work has resulted in the characterization of a new immunostimulatory â -(1 Æ3,1 Æ6)-glucan from the marine diatom *Chaetoceros mülleri[5*].

In two first feeding experiments the *C. mülleri* glucan and commercial immunostimulants based on yeast and alginates were administered orally to cod larvae. The results show that the *C. mülleri* glucan induced higher survival and growth of the larvae during the critical weaning phase from live feed to formulated diet.

Our results indicate that the chrysolaminaran from *C. mülleri* is a highly potent immunostimulant, and represents a new pharmaceutical product from a marine bioresource which is independent of seasonal variations. *C. mülleri* is easily cultivated and experiments have shown that a stable product may be obtained without excessive control of the cultures[6] which is an important factor simplifying large scale production. These findings indicate that the commercial potential of our findings is high.

1. Raa, J., *The use of immunostimulatory substances in fish and shellfish farming.* Reviews in fisheries Science, 1996. 4(3): p. 229-288.
2. Sakai, M., *Current research status of fish immunostimulants.* Aquaculture, 1999. **17**2: p. 63-92.
3. Bohn, J.A. and J.N. BeMiller, *(1->3)-beta-D-glucans as biological response modifiers: A review of structure-functional activity relationships.* Carbohydrate Polymers, 1995. **2**8(1): p. 3-14.
4. Granum, E., *Metabolism and function of b-1,3-glucan in marine diatom*s, in *Department of Biotechnology*. 2002, NTNU: Trondheim. p. 48.
5. Størseth, T.R., K. Hansen, J. Skjermo, and J. Krane, *Characterization of a beta-D-(1 -> 3)-glucan from the marine diatom Chaetoceros mülleri by high-resolution magic-angle spinning NMR spectroscopy on whole algal cells.* Carbohydrate Research, 2004. **33**9(2): p. 421-424.
6. Størseth, T.R., K. Hansen, K.I. Reitan, and J. Skjermo, *Structural characterization of -D-(1'3)-glucans from different growth phases of the marine diatoms Chaetoceros mülleri and Thalassiosira weissflogii.* Submitted Carbohydrate Research, 2004.

By-products of cultured blue mussel: BioGlue (working title)

Tone Rasmussen

SEA ECO AS, Havnepromenaden 11, 9405 HARSTAD

A new and highly efficient water and biocompatible biological glue has been developed by Biopolymer Products AB.
The product is now tested and test results so far are positive due to its uniqe structure and strength.
Main areas for use is plastic and eye surgery, the developers have also registered an increased interest from the developing nanotechnology -industri.
Further development of a more efficient production process for the glue is now in progress in cooperation with SEA ECO AS in Harstad.

Making money from marine biotechnology: marrying scientific and business strategies

Dr J. Douglas McKenzie

Integrin Advanced Biosystems Ltd, Marine Resource Centre, Barcaldine, Argyll, Scotland PA37 1SE

Tel 44 1631 720748, Fax 44 1631 720590, E-mail: dmck@integrin.co.uk
www.integrin.co.uk

Marine biotechnology offers an unsurpassed variety of opportunities to develop new products, ranging from new energy sources, environmental technologies, through pharmaceuticals, biomedical applications and on to food. The sheer diversity brings with it a problem: what do you decide to focus commercial development on?

At Integrin, we have been considering this question for the past four years. Running a company as opposed to a research group rapidly brings a focus because the fundamental rule is that you must be able to make money within a defined time period and at a sufficient rate of return to attract investment. Marine biotechnology is no exception to this. In establishing whether or not a particular area of marine biotechnology can deliver on this fundamental priority then the starting point is a market study. Who would buy the products or service derived from marine biotechnology? How many customers would the product or service have? What would they be willing to pay? What is the competition and how would they react to a new product or service invading their niche? The next stage is to look at the technical and commercial development needed to launch the product or service. How long will it take? How much money will it cost? How will the development be funded and what will the funders want for their money?

From the market and technical analyses it quickly becomes obvious that most areas of marine biotechnology currently do not currently represent genuine commercial opportunities either because their markets are too small or the time to develop the technologies into commercial products is too long.

Integrin's journey of transition from an academic research group into a hardened commercial entity will be used as a case study in how we can develop more successful businesses in marine biotechnology.

BIOPROPECTING ON METAZOAN – THE CASE OF *CALANUS*

Kurt Tande

CALANUS AS

Tromsø Norway

Three years ago the goal was set to develop the company to be a pioneer on the market with products based on the marine copepod *Calanus finmarchicus* within sectors like food ingredients flavour and dietary supplements. On the basis of research based knowledge the company CALANUS AS shall be the leading company on harvesting, utilization and sale of products from *Calanus*. In 2004 the first products in food ingredients flavour has been introduced at the market, as well with tests on dietary supplements. The company has newly released the first versions of feed for fish larvae and adult fish.

Calanus is the resource basis for several commercial important marine species, and has a chemical composition, which is a complete dietary support for both fish and humans. *Calanus* has never been exploited as a basis for food or feed, and it is therefore a need to know the nutrient characteristics in depth, both through chemical analysis and by feeding experiments. The stock of *Calanus* is estimated to 200 – 400 million tons wet weight for the Nordic Seas, and only from 10 to 15 % of the annual production is being utilized and incorporated further up in the food chain.

Data shows that *Calanus* contains essential metals, fatty acids, amino acids, anti oxidants (i.e. astaxanthin), and a wide variety of important vitamins and minerals.
Waxes are a unique lipid and special component for *Calanus*, and are not found to that extent in other organisms. Waxes have biological and chemical properties, which are relevant for several areas. Such components can be used in technical products, in cosmetics, as dietary supplements, and chemical industry. Since the copepod is found in the base of the food chain and is adapted to a life in the free water masses, this form does not contain toxins. This further increases its potential value in the market.

CALANUS AS possesses knowledge on resource biology, management and harvesting of this copepod. The company has already developed cost efficient harvesting methods and has developed a unique first generation trawling concept. The company has further in depth knowledge of the market potential for new products that can be generated from *Calanus*. Relevant applications and products to day are: - bioactive ingredients, -attractants, - special feed for animals and fish, - food ingredients flavour, - cosmetics and - chamicals

The contribution conveys important milestones in the short history of the company, status today and as well as perspectives for the future.

Utilizing marine compounds in environmental and food safety diagnostics applications

Anders Goksøyr

Biosense Laboratories AS & Department of Molecular Biology, University of Bergen, Bergen, Norway

e-mail: anders@biosense.com

Many proteins produced by aquatic organisms can be utilized in diagnostic applications, either as biomarkers representing a response in the living organisms to an environmental challenge such as pollution, or as binding proteins that can be useful in assays for toxic compounds.

In the first (biomarker) case, knowledge of the response to pollutants in the organism under study is important, e.g. range of compounds producing the response, biotic and abiotic factors affecting the response, gender and species differences etc. To be able to use the protein as a standard in the diagnostic assay, it is also important to obtain the protein under study in a highly stable and reproducible form. Finally, the use of the biomarker needs to be validated under realistic conditions in field and laboratory studies. Here I will present our work to establish the vitellogenin response in fish as a test for endocrine disrupting compounds in the environment.

In the second case, the targets of toxic action, e.g. receptor sites for toxin binding, are often good candidates for binding proteins in receptor-ligand assays. Again, basic knowledge of the molecular biology of the receptor protein, its ligand-binding properties, factors affecting binding etc., is a necessary fundament before developing an assay. For some applications, the protein can be extracted from its source in a stable and functional form, but for others it may be necessary to make recombinant versions of the receptor, which then can also be functionalized in other ways by adding tags, epitopes or reporter moieties. Work is ongoing to establish an assay for dioxins in food based on the salmon (*Salmo salar*) dioxin (Aryl hydrocarbon, Ah) receptor, and to develop an assay for diarrhetic shellfish poisoning (DSP) in mussel, based on protein phosphatase 4 cloned from blue mussel (*Mytilus edulis*). Results and experiences from this work will be presented.

The R&D work at Biosense Laboratories AS is supported by grants from the Norwegian Research Council (NFR), Innovation Norway (SND) and the EU FP5 (EASYRING) and FP6 (BIOTOX).

Abstracts ~ posters

The Norwegian Structural Biology Centre - NORSTRUCT

Vibeke Os, NORSTRUCT

Department of Chemistry, Faculty of Science, University of Tromsø

Department of Molecular biotechnology, Faculty of Medicine, University of Tromsø

Department of Marine Biotechnology, Norwegian College of Fishery Science

NORSTRUCT is a national service and competence centre in structural biology, located at the University of Tromsø. The Centre aims at establishing high throughput structural biology facilities of high international standard for structure determination and 3D analysis of biologically active macromolecules. NORSTRUCT is established through the Norwegian functional genomics initiative (FUGE) and it is financed by the FUGE-program and the University of Tromsø.
Activities will be divided between local and external projects and the Centre seeks to streamline the processes starting from recombinant protein production to 3D structure determination using X-ray crystallographic techniques. Through participation in external projects, the Centre will offer the Norwegian molecular biology research community services, competence and instrumentation in a broad range of techniques in addition to serve as a link to international large-scale facilities such as the European Synchrotron Radiation Facilities (ESRF) in Grenoble.

Marbio - A platform for screening and exploration of unique bioactivities in marine organisms

Jeanette Hammer Andersen

University of Tromsø

Marbio is a medium/high-throughput analytical platform with the aim of bioprospecting a large number of marine organisms from Arctic and sub-Arctic regions for potential drugs and/or lead compounds. The platform has the capacity to collect, extract and purify molecules with unique bioactivities within the most important drug areas, including molecules with anti-bacterial, antiviral, anticancer and immunostimulatory or anti-inflammatory action, in addition to various enzyme activities. The Marbio platform will have a close cooperation with the new marine biobank (Marbank), which will be established "next door" to Marbio.
Marbio is based on a tight interaction of the combined resources from several scientific groups at the University of Tromsø, University Hospital of North-Norway and industry.

Envision Software Package for the Concerted Study of Biological Data.

Chris Fenton

Dep. Molecular Biotechnology, Institute of Medical Biology,

University of Tromsø

Mathematical significance is not biological significance. Therefore, biological data must be interpreted, coordinated, and presented to skilled experimentalists, and bioinformatititions is such a way that they can validate, or derive hypotheses. In order to correctly interpret biological data researchers must often take several aspects into account; structure, homology, phylogeny, etc. The difficulty lies in coordinating all the data. The coordination of different views, and analyses is the main goal of the Envision software package. Hopefully aiding in the interpretation and elucidation of biological data.

Novel alternative spliced factors affecting muscle growth and reproduction in Atlantic halibut

Andersen, Ø[1], Kobayashi, T[1], Wiborg Dahle SV[2], Galloway, T[2]

[1]AKVAFORSK, P.O.Box 5010, 1432 Ås-NLH

[2]Dept. of Biology, NTNU, 7491 Trondheim

Growth and reproduction are regulated by both environmental and genetic factors, making it possible to control these traits in aquaculture species. In Atlantic halibut, both the larval muscle growth and the asynchronous oocyte maturation are largely unknown processes. The myogenic regulatory factors (MRFs) MyoD and myogenin were cloned from halibut skeletal muscle to study the molecular mechanisms of muscle development. The duplicated genes encoding MyoD1 and MyoD2 have apparently adopted different functional roles in the myogenesis and metamorphosis of the halibut larvae. A novel way of processing the MyoD2 pre-mRNA and the alternative splicing of myogenin pre-mRNA generated C-terminal truncated isoforms of both transcription factors with currently unknown functions.

To address the regulation of oocyte growth and batch spawning in halibut the gonadotropic hormones and their receptor FSH-R and LH-R were cloned. The intact receptors consisted of a long extracellular hormone binding domain, seven transmembrane domains, and a short C-terminal intracellular domain. Additional C-terminal truncated variants of FSH-R lacking the signal transducing domain were isolated, and their potential inhibitory roles are now being examined. In summary, whereas the identified factors involved in growth and reproduction are well conserved in halibut, additional isoforms with potential novel functions are generated as the result of alternative pre-mRNA processing.

Purification of three bioactive compounds from a marine diatom species

Linn Oftedal[1], Rosemary T. Coyne[1], Siv K. Prestegard[2], Kaja Skjærven[1], Einar Solheim[3], Khanh Kim Dao[1], Frode Selheim[1], Lars Herfindal[1], Gjert Knudsen[2], and Stein Ove Døskeland[1]

[1]Cell Biology Research group, Section of Anatomy and Cell Biology, Department of Biomedicine, University of Bergen

[2] Section of Microbiology, Department of Biology, University of Bergen

[3]PROBE, Proteomics Unit at University of Bergen

In order to identify natural products from marine organisms capable of inducing apoptosis in cancer cells, benthic marine diatom species were screened for content of bioactive compounds. Even though the diatoms are the largest group of microalgae in the sea, few bioactive compounds have so far been isolated from this diverse group. The most known toxin is the neurotoxin domoic acid, which is produced by a small number of diatom species.

A marine diatom species, B58, was found to produce two distinct apoptosis-inducing compounds. Their apoptogenic activity could not be caused by domoic acid, since this amino acid derivative has no effect on the cell types tested.

An advanced purification protocol for these two distinct bioactive compounds, *Apo-58A* and *Apo-58B*, was developed. This method consist of water-extraction, solid phase extraction and both semipreparative and analytical high performance liquid chromatography.

Transmission electron microscopy revealed that Apo-58A induced autophagic cell death in rat promyelogenous leukaemia cells. In addition both *Apo-58A* and *Apo-58B*, modulated activation of blood platelets. *Apo-58A* blocked both platelet aggregation and α-granule secretion, whereas *Apo-58B* inhibited only α-granule secretion. A third bioactive compound was also isolated and found to be a potent activator of blood platelets.

The molecular weight of the compounds was determined by mass spectrometry. One of these compounds Apo-58A was proven, by the use of mass spectrometry and enzymatic cleavage, to be adenosine.

We have also purified a novel apoptogen, distinct from those described above, from another benthic marine diatom species (L.Herfindal et al. MS in prep.)

In conclusion marine benthic diatom species is a new source for novel apoptogenic compounds.

Screening leukaemia cell lines for apoptosis inducing compounds extracted from marine and freshwater cyanobacteria

Kaja Skjærven1, Linn Oftedal1, Lars Herfindal1, Kaarina Sivonen2, Bente Edvardsen3, and Stein Ove Døskeland1

[1]Cell Biology Research group, Section of Anatomy and Cell Biology, Department of Biomedicine, University of Bergen, Norway

[2]Cyanobacteria group, Department of Applied Chemistry and Microbiology, University of Helsinki, Finland

[3]NIVA Culture Collection, Norwegian Institute for Water Research (NIVA), Oslo, Norway

Multiple cyanobacterial strains were investigated as sources of natural compounds capable of inducing apoptosis. Some of the strains were marine species collected from the Baltic Sea and some strains were freshwater species collected from Norwegian lakes.

Dried cyanobacteria biomass was sequentially extracted to separate potential bioactive compounds. Water, methanol and dichloromethane were used in a combination to separate the compounds into fractions according to their polarity. The extracts were tested on two leukaemia cell lines (IPC-81 and HL60) and one lymphoblastoma cell line (Jurkat-T). The species from the Baltic Sea have also been tested on primary rat hepatocytes, human fibroblasts and human thrombocytes.

Apoptogenic inducing activity was observed in both polar and nonpolar fractions, and some species contained more than one bioactive compound. Certain species produce bioactive compounds that exclusively kill leukaemia cells. One species produced a compound that had an apparent anti-apoptotic effect, and the compound has been completely purified. It was also found that the cyanobacteria induce different apoptotic phenotypes in the cell, and are therefore certain to be of interest as cell biology tools. In collaboration with Haukeland University Hospital, Bergen, we will test anti-leukaemic compounds in a newly established rodent leukaemia model. We conclude that cyanobacteria are a rich source of apoptosis inducing compounds, and it is hoped that some of these will prove to be useful as therapeutic pharmaceuticals.

ANTIBACTERIAL ACTIVITY IN CRUDE EXTRACTS OF MARINE MACROALGAE

I. Salvesen1, O.G. Brakstad2, M. Givskov3, G. Johnsen4, M. Skindersø3, and T. Størseth1

[1]SINTEF Fisheries and Aquaculture, Dept of Bioressources, N-7465 Trondheim, Norway

[2]SINTEF Materials and Chemistry, Dept of Marine Environmental Technology, N-7465 Trondheim, Norway

[3]Technical University of Denmark, Centre for Biomedical Microbiology, Biocentrum, Bldg 301, DK-2800 Lyngby, Denmark

[4]NTNU – Norwegian University of Science and Technology, Trondhjem Biological Station, Bynesveien 46, N-7491 Trondheim, Norway

The potential of marine organisms as a resource to chemicals with novel molecular structure is well documented. Marine macroalgae produce an array of bioactive metabolites with antibacterial and antifouling activity that may have possible biotechnological applications, but more knowledge is needed to understand which factors trigger this production. Metabolites produced by the algae may kill or inhibit bacterial growth and/or they may interfere with *quorum sensing* and the expression of specific bacterial characteristics important for fouling and virulence. In the present study, antibacterial activity in marine macroalgae from a temperate fjord that experiences pronounced variations in temperature, light and wave action have been studied.

Macroalgae were collected monthly at Trondhjem Biological Station (TBS) from February 2002-2003 or at low tide at various locations in the Trondhjemsfjord (63°N) in April-June 2002-2004. The location at TBS is exposed to wave and tidal currents and is characterized by large rocks. An underwater station was established to monitor key environmental parameters such as salinity, temperature, depth and irradiance (SAIV STD/CTD model SD204, LI-COR LI-1400 irradiance data logger). Antibacterial activity in crude extracts was evaluated as growth inhibition (against a selection of Gram negative/positive bacteria, human and fish pathogens) by the agar diffusion method and/or as *quorum sensing* inhibition (QSI) in selector systems based on the *lux*-system from *Vibrio fisheri*.

All three main groups of macroalgae (*Phaeophyceae, Rhodophyceae* and *Chlorophyceae*) produced metabolites that inhibited growth of both terrestrial and marine bacteria. A few algae were shown to inhibit and/or even induce *quorum sensing*. *Quorum sensing* induction could be an algal strategy for stimulating early release of virulence factors, and thereby triggering the defence mechanisms of the algae, before bacterial populations reach a critical harmful level. No clear relation was found between variations in key environmental parameters studied here and antibacterial activity, and the production of growth inhibitory metabolites was relatively stable throughout the year. Further chemical characterization of bioactive compound(s) is in progress.

This study was supported by grants from the Norwegian Research Council (project number 146633/120 and 143450/140).

The antibacterial effect of a polyhydroxylated fucophlorethol from the marine brown alga *Fucus vesiculosus*

E. Sandsdalen1, T. Haug2, K. Stensvåg2 and O. Styrvold2

1) Fiskeriforskning, P.O. Box 6122, N-9291 Tromsø, Norway
2) Department of Marine Biotechnology, Norwegian College of Fishery Science, University of Tromsø, Norway

The evolution of antibiotic resistant pathogenic bacteria has stimulated the search for new anti-microbial agents form natural sources. Much of nature remains to be explored, particularly the marine and microbial environments and the interplay of these two sources.

The algae belong to a group of organisms that has enormous ecological importance and represents a significant proportion of the world`s biodiversity. The main class of commercially valuable algae products is the algal polysaccharides, but algae also produce a range of unique secondary metabolites. Several metabolites with anti-microbial and cytotoxic activities have been isolated and characterized from brown algae.

We have now isolated and characterized a polyhyroxylated fucophlorethol with antibacterial effect from *Fucus vesiculosus*.

EXTRACTS FROM THE MARINE ALGAE *Phaeocystis pouchetii* AFFECT MITOTIC CELL DIVISIONS IN SEA URCHIN EMBRYOS.

E. Hansen[1], A. Ernstsen[2], Y. Even[3], A.M. Geneviève[3] and H. C. Eilertsen[1].

1 Dept. of Aquatic BioSciences, NFH, University of Tromsø, N-9037 Tromsø, Norway

2 Dept. of Biology, Faculty of Science, University of Tromsø, N-9037 Tromsø, Norway

3 Laboratoire Arago, CNRS-UMR 7628 Universitè de Paris 6, F-66650 Banyuls-sur-Mer, France

The marine microalga *Phaeocystis pouchetii* is a major component of the phytoplankton spring bloom in northern and temperate waters, and it is suspected to produce some compound responsible for reducing growth, fecundity and survival of other marine organisms. In order to gain more knowledge of the extent of the toxic effect and nature of this putative toxin, we tested the effect of extracts from *P. pouchetii* cultures on growth and development of sea urchin embryos. We found that organic extracts from the algae culture media blocked cell divisions in embryos of the sea urchin *Sphaerechinus granularis*. Using immunofluorescent staining of α-tubulin subunits we found that extracts from *P. pouchetii* cultures inhibited formation of new microtubules and induced rearrangements of existing microtubular arrays. Bioassay-guided HPLC separation of the organic extract was performed to trace the toxic components, and analysis by GC-MS showed that the α,β,γ,δ-unsaturated aldehyde 2-*trans*-4-*trans*-decadienal was present in the extracts. We found that a commercial standard of 2-*trans*-4-*trans*-decadienal and organic extracts from *P. pouchetii* had similar effects on the early events of sea urchin embryogenesis: in both cases pronuclear migration and fusion and DNA replication were blocked and aberrations in mitotic spindles were induced. Furthermore, we found that the G_2-M phase promoting complex cyclin B/Cdk1 was inactivated in nondividing eggs incubated in 2-*trans*-4-*trans*-decadienal, despite the accumulation of cyclin B. We conclude that 2-*trans*-4-*trans*-decadienal was responsible for the cytotoxic effects of the *P. pouchetii* extracts.

Purification and characterization of proline-rich and cysteine-rich antibacterial peptides from the haemocytes of the spider crab, *Hyas araneus*

Tor Haug [a], Klara Stensvåg[a], Terje Vasskog [b], Erling Sandsdalen [c], Olaf B. Styrvold [a]

[1]Institute of Marine Biotechnology, Norwegian College of Fishery Science, University of Tromsø, Norway,

[2] Institute of Pharmacy, Faculty of Medicine,University of Tromsø, Norway,

[3] Norwegian Institute of Fisheries and Aquaculture, Tromsø, Norway,

E-mail:olafs@nfh.uit.no

Antimicrobial peptides are important components of the innate immune system of both vertebrate and invertebrate animals.[1] In a previous study we investigated the antibacterial activity in different body-parts of four marine crustacean decapods. Dried samples were extracted with 60% acetonitril, containing 0.1% (v/v) trifluoroacetic acid, and further extracted and concentrated on C_{18} cartridges. Eluates from the solid phase extraction were then tested for antibacterial activity.[2] Based on results from this study, several antibacterial peptides were isolated and characterised from the haemocytes of the small spider crab, *Hyas araneus*. Two of the peptides were shown to be novel members of the proline-rich family of antimicrobial peptides. Both peptides consisted of more than 25% proline and their monoisotopic masses were 2953 and 4342 Da, respectively. It is composed of two domains, a proline/arginine-rich N-terminal domain and a C-terminal domain containing two disulphide linkages. In a liquid growth inhibition assay both the native polypeptides and partial N-terminal synthesised proline-rich sequences were shown to be active against Gram-positive and Gram-negative bacteria in μM concentrations.

1. Zasloff, M., *Nature*, **2003**, 41, 389-395.
2. Haug, T., A. K. Kjuul, K. Stensvåg, E. Sandsdalen, and O. B. Styrvold. *Fish and Shellfish Immunology*, **2002**, 12, 371-385.

Molecular cloning and characterization of crustin-like, cysteine-rich Defence polypeptides from the spider crab, *Hyas araneus* and the red king crab, *Paralithodes camtschatica*

Klara Stensvåg [a], Tone Mari Rode[a], Guro Strandskog[a], Tor Haug[a], Olaf B. Styrvold[a]

[a]Department of Marine Biotechnology, Norwegian College of Fishery Science, N-9037 University of Tromsø, Norway,

E-mail: klaras@nfh.uit.no

Antimicrobial peptides are widespread and found in all living species studied. They are important components in the natural defence against microbial infection. Investigations by Haug et al.[1] on crustaceans have demonstrated antibacterial, lysozyme and haemolytic activities in various organs and tissues. Some of the active compounds from the haemocytes from the spider crab (*Hyas araneus*) have been isolated and we have purified, from unchallenged individuals, two antimicrobial polypeptides, named crustin Ha1 and crustin Ha2.

The native polypeptides are rich in cystein, have monoisotopic masses of approximately 10.1 kDa, and display antimicrobial activity against both Gram positive and Gram negative bacteria.

Primers for PCR were chosen on the basis of partial N-terminal peptide sequences, and Crustin Ha cDNA sequence analysis indicated that it is processed from a precursor containing a signal peptide. In this work, two full-length cDNA sequences have been isolated. The polypeptide isolated from *H. araneus* are identified as homologues to carcinin, an antimicrobial polypeptide previously isolated from *Carcinus maenas*, and putative antimicrobial peptides from *Litopenaeus vannamei* (*Lv*1) and *Litopenaeus seriferus* (*Ls*1)[2-4].

A full length cDNA sequence of an homologue to crustin Ha was isolated from a cDNA library from the hemocytes of the red king crab, *Paralithodes camtschatica*. The mature peptide isolated from *P. camtschatica* is yet to be isolated.

References

1. Haug T., A.K. Kjuul, K. Stensvåg, E. Sandsdalen, O.B. Styrvold, *Fish & Shellfish Immunology*, **2002**, 12, 371-385.
2. Bartlett T., C.B.J. Cuthbertson, E.F. Shepard, R.W. Chapman, P.S. Gross, G. Warr. *Marine Biotechnology*, **2002**, 4, 278-293.
3. Relf J.M., J.R.S. Chisholm, G.D. Kemp, V.J. Smith, *European Journal of Biochemistry*, **1999**, 264, 350-357.
4. Smith V.J., J.R. Chisholm, *Advances in Cirrhosis, Hyperammonemia, and Hepatic Encephalopathy*, **2001**, 484, 95-112.
5. Supungul, P., et al., *Identification of Immune-Related Genes in Hemocytes of Black Tiger Shrimp (Penaeus monodon).* Mar Biotechnol (NY), 2002. **4**(5): p. 487-94.
6. Chen, J.Y., C.Y. Pan, and C.M. Kuo, *cDNA sequence encoding an 11.5-kDa antibacterial peptide of the shrimp Penaeus monodon.* Fish & Shellfish Immunology, 2004. **16**(5): p. 659-64.

Increased molecular flexibility plays a central role in cold-adaptation of Uracil DNA Glycosylase

Magne Olufsen, Bjørn Olav Brandsdal, Elin Moe and Arne O. Smalås

The Norwegian Structural Biology Centre, University of Tromsø

Uracil DNA glycosylase (UDG) is a DNA-repair enzyme and is involved in the base excision repair pathway (BER) removing misincorporated uracil from DNA. Atlantic cod UDG (cUDG), which is a cold-adapted enzyme, has been found to be up to ten times more catalytically active in the temperature range 15 to 37 °C compared to the warm-active human counterpart[1]. The increased catalytic activity and reduced temperature stability of cold-adapted enzymes compared to their mesophilic homologues are partly thought to be caused by an increase in the structural flexibility [2]. However, no direct experimental evidence supports the proposal of increased flexibility of cold-adapted enzymes. Molecular dynamics (MD) simulations have been used to calculate the structural flexibility of UDG. The results from these simulations show that an important loop involved in DNA recognition (Leu272 loop) is the most flexible part of the cUDG structure, and that the human counterpart has much lower flexibility in the Leu272 loop. The flexibility in this loop correlates well with the experimental k_{cat} values [3].

[1] Lanes et al. (2000). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 127(3): 399-410.
[2] Georlette et al. (2004). Fems Microbiology Reviews 28(1): 25-42.
[3] Moe et al. in press.

Structural basis for S1-S4 specificity in two enzymes of the ProteinaseK family.

Larsen, A.N.[1], Helland, R.[2], Gjellesvik D.R.[3] and Willassen N.P.[1]

[1] Department of Molecular Biotechnology, Faculty of Medicine, University of Tromsø, Norway, [2] Department of Chemistry, Faculty of Science, University of Tromsø, Norway, [3] Biotec Pharmacon ASA

A protease from a *Serratia* species, sharing ca 40% identity with ProteinaseK of the subtilisin family, has been isolated, cloned, expressed and now the crystal structure is determined to 1.8 Å and an R-factor of 14.6% and R-free of 18.0%.
The catalytic efficiency of the two enzymes towards various tri- and tetrapeptide substrates has been compared. The two enzymes display slightly different substrate specificity, and the catalytic turnover (k_{cat}) is higher for the new protein than ProteinaseK, but the substrate binding (K_M) is poorer.

The Vibrio salmonicida genome project

Paulsen, S.M.[1], Ræder, I.L.U.[1], [1]Pedersen, H.L.[1], Paiva, H.H.[2], Hjerde, E.[1], Jørgensen T.[3], Smalås, A.[4], Parkhill, J.[5] , Billaut, A.[6], Espelid, S.[1] & Willassen, N.P.[1]

[1]Department of Molecular Biotechnology, Institute of Medical Biology, University of Tromsø, Norway, [2]Faculty of Pharmaceutical Science of Ribeirão Preto, University of São Paulo, Brazil, [3]Department of Molecular Biotechnology, Norvegian College of Fishery Science, UiT, Norway [4]Department of Chemistry, Faculty of Science, UiT, Norway [5]The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK, [6]Molecular Engines Laboratories, 75011 Paris, France,

Vibrios are Gram-negative γ-protobacteria that is ubiquitous in marine and estuarine environments. Bacteria of the Vibrionaceae family, which shows a comma-shaped microscopic appearance and polar flagella appendage, are mostly aquatic inhabitants that require NaCl for optimal growth. *Vibrio salmonicida* is the causative agent of cold-water vibriosis in farmed Atlantic salmon (Salmo salar). Cold-water vibriosis is primarily a disease of Atlantic salmon, but has also been encountered in other sea farmed fish species, where it elicits tissue degradation, haemolysis and sepsis. The success of *V. salmonicida* as a pathogen is somewhat curious since the organism seems not to have an abundance of classic virulence factors. However, as a pathogen, its genome must harbour a number of genes encoding proteins and enzyme systems able to cope with an array of host defence molecules.

Due to its property as a psychrophilic organism having two different life cycles; in the environment and in the fish body, its genome is require to harbour array of genes encoding proteins of great difference in properties. In that respect, *V. salmonicida* is regarded as an interesting source of new proteins. Furthermore, what also makes this organism worth studying is that it harbours a number of DNA replicons indicating a gene organisation more complex compared with the *Vibrio* species so far sequenced.

As a part of the complete genome sequencing of *V. salmonicida*, the genome of has been made available for effective screening with specific DNA probes. A BAC (bacterial artificial chromosome) library with an insert size of 70 kb *V. salmonicida* DNA has been arrayed on membranes. A number of genes have been identified, cloned and expressed for further structural functional studies.

The genome-sequencing project is carried out following both an ordered BAC clone strategy and whole genome shotgun strategy. The BAC library has been end-sequenced and a number of selected BAC clones have been sequenced to six times coverage, assembled and annotated. The whole genome sequencing will be carried out in collaboration with The Pathogen Sequencing Unit at The Wellcome Trust Sanger Institute.

Functional studies are carried out as extensive proteome analysis combining two dimensional gel electrophoresis and mass spectrometry. Proteome comparison of *V. salmonicida* cultured under a range of *in vitro* conditions has been performed. Protein spots are identified searching databases with the obtained peptide mass fingerprints. When peptide mass fingerprint search are unsuccessful or ambiguous *de novo* peptide sequencing is required.

This genome & proteome analysis is the starting point for in-depth understanding of *V. salmonicida* behaviour in different environments.

Sequencing and annotation of two cryptic plasmids from the fish pathogen *Vibrio salmonicida*.

Marit Sjo Lorentzen[1], Erik Hjerde[1] and Nils-Peder Willassen[1,2]

[1]Department of Molecular Biotechnology, Institute of Medical Biology, [2]The Norwegian Structural Biology Centre (NORSTRUCT), University of Tromsø, 9037 Breivika, Norway

Correspondence should be addressed to N. P. Willassen, Department of Molecular Biotechnology, Institute of Medical Biology, University of Tromsø, N-9037 Tromsø, Norway. Tel.: +47 77 64 46 51, Fax: +47 77 64 53 50, E-mail: nilspw@fagmed.uit.no

The gram-negative *Vibrio salmonicida* is responsible for cold-water vibriosis of farmed Atlantic salmon (*Salmo salar*), cod (*Gadus morhua*) and rainbow trout (*Oncorhynchus mykiss*). The bacterium causes tissue degradation, haemolysis and sepsis *in vivo*. In this study the two smallest plasmids, pVS54 and pVS43 from *V. salmonicida* strain LFI1238 were sequenced and analyzed. Plasmid pVS54 has a total G + C content of 38.1% and consists of 5360 bp, constituting three open reading frames (ORF's), whereas pVS43 has a total G + C content of 35.6% consists of 4327 bp, also constituting three ORF's. The analyses indicate that both plasmids are replicating by the theta mode of replication, and pVS43 seems to belong to the iteron-containing class while pVS54 have origins of replication without iterons.
Both plasmids carry genes encoding acetyltransferases and genes for plasmid replication. The acetyltransferases may be important for the bacteria-host interaction.

Proteomic studies of *Vibrio salmonicida*

Ræder I.L.U[2]., Pedersen H.L[3]., Paulsen S[1]., Jørgensen T[3,4]., Willassen N.P[1,4]., Smalås A[2,4].

Department of Molecular Biotechnology[1], Department of Chemistry[2], Norwegian College of Fishery Science[3], NORSTRUCT (The Norwegian Structural Biology Centre)[4], University of Tromsø, Norway

Effects of different cultivation conditions on the expression pattern of *Vibrio salmonicida* proteins were investigated by western blot and 2D gel electrophoresis. With different growth stimulants, altered expression patterns of unstimulated bacterial cells have been discovered. Currently, stimulants like salmon mucus, hydrogen peroxide, iron chelator and different temperatures have been used. Also, immunoblotting of the gels with immunsera from salmon infected with *V. salmonicida* strain LFI315 was performed to identify proteins potentially involved in virulence.

Hypothetical proteins from *Vibrio Salmonicida* genome: strategies for target selection, cloning and expression

Yang N.*§, Espelid S.¶, Willassen, N.P.¶, Smålås, A.O.*

***Norwegian Structural Biology Centre, Faculty of Science, University of Tromsø, Norway.**

¶Institute of Medical Biology, University of Tromsø, Norway

§ Corresponding author: email: *nannan@chem.uit.no*

Around 9% of *Vibrio Salmonicida* (an atlantic salmon pathogen and gram negative microorganism) genome is sequenced and annotated. The sequence data contains information for around 400 proteins. Those proteins are classified into four categories, (1) proteins with known function and known three-dimensional structure, (2) proteins with known function but unknown three-dimensional structure, (3) conserved hypothetical proteins, and (4) hypothetical proteins (Figure 1). The last two categories were included in the current project. Among the conserved hypothetical proteins, some will be Vibrio/pathogen specific. Those Vibrio/pathogen specific conserved hypothetical proteins and hypothetical proteins which is *Vibrio Salmonicida* specific were further analyzed by using bioinformatic tools including primary and secondary structure analysis and tertiary structure prediction. The sequences that contain either coiled coil region or transmembrane segments were excluded due to difficulties in crystallization. After sequence analysis, 10 candidate proteins were selected for further work, that is, cloning, expression, characterization, purification crystallization and structure determination. Two of these proteins were selected for pilot experiment e.g. cloning, expression, purification, crystallization and structure determination. One of the proteins was expressed in *E. coli* strain BL21-AI and strain BL21(DE3) Codon plus by using gateway technology with optimal protein production temperature 22°C and strain BL21-AI produced most protein.

DNA adenine methylase and RecJ exonuclease from *Vibrio salmonicida*

Niiranen L., Espelid S., Smalås A. and Willassen N.P.

Department of Molecular Biotechnology, Institute of Medical Biology and

Norwegian Structural Biology Centre

Previous studies have shown that DNA adenine methylase (Dam) negative strains of *Salmonella typhimurium* are essentially avirulent. Our goal is to find out if also the *Vibrio salmonicida* Dam is involved in virulence. Based on structural and functional studies the characteristics of possible cold adaptation of the DNA modifying enzymes Dam and RecJ exonuclease will be described.

The native forms of Dam and RecJ could not be overexpressed in *E. coli* in soluble form, so an expression screen was made with seven different N-terminal tags, two host strains and two culture temperatures. Soluble Dam and RecJ fusion proteins with MBP tag were purified by affinity chromatography and gel filtration and were found to be active. A new assay method based on fluorescence was developed for RecJ exonuclease.

Are lysines important for cold adaptation of *Vibrio salmonicida* nuclease?

B. Altermark[1], E. Moe[1], A.O. Smalås[1] & N.P. Willassen[1,2]

[1]Norwegian Structural Biology Centre and [2]Department of Molecular Biotechnology, Faculty of Medicine, University of Tromsø

Vibrio salmonicida is a psychrophilic bacterium that causes cold water vibrosis (hemorraghic syndrome or Hitra disease) in Atlantic salmon (*Salmo salar*) and Atlantic cod (*Gadus morhua*). The gene encoding a periplasmic nuclease, orthologous to endonuclease I of *Escherischia coli*, is isolated from this bacterium. The *V. salmonicida* nuclease is compared to nucleases from other related bacteria in order to find features that may be involved in possible cold adaptation. The results from sequence comparison show that there is a drastic increase in Lysines among the cold-adapted species compared to the warm adapted ones. In order to differentiate between random mutations, and mutations due to low temperature adaptation, nucleases from different vibrios have been studied and compared. The phylogenetic relationship between the bacteria has also been analysed. The gene for *V. salmonicida* nuclease has been expressed and purified. Enzyme properties will be compared against the same nuclease in the human pathogen *V. cholerae*, when it is produced. Crystallisation of the *V. salmonicida* nuclease is in progress, and hopefully the X-ray structure will be solved both for the *V. salmonicida* and V. cholerae nuclease. In a recent paper the structure of *V. vulnificus* nuclease was presented. This bacterium is a human pathogen and mesophilic. A model of *V. salmonicida* nuclease has been constructed using *V. vulnificus* nuclease as template. When compared, the *V. salmonicida* nuclease reveals a more positively charged surface than the mesophilic *V. vulnificus* nuclease. A more positively charged nuclease may have higher affinity to the negatively charged nucleic acids, and it may be that the increase in lysines found in *V. salmonicida* nuclease and other cold loving vibrios is a compensation for slow reaction rates at low temperature.

List of participants

Ahmad	Rafi	rahmad@fagmed.uit.no	PhD Student	**University of Tromsø** Dept. of Medical Biotech Inst. of Med. Bio Faculty of Medicine N-9037 Tromsø Norway
Altermark	Bjørn	bjorna@chem.uit.no	Research Fellow	**University of Tromsø** Department of chemistry 9037 Tromsø Norway
Amlie	Thor	thor.amlie@pil.no	Director	**Norwegian Bioindustry Association** P.O. Box 5487 Majorstuen 0305 Oslo Norway
Andersen	Jeanette	jeanettea@nfh.uit.no		**University of Tromsø/Marbio** Øvre Lysthus 9037 Tromsø Norway
Andersen	Øivind	oivind.andersen@akvaforsk.nlh.no	Professor	**AKVAFORSK** P.O.Box 5010 1432 Ås Norway
Arnesen	Jan Arne	jan-arne.arnesen@fiskeriforskning.no	Scientist	**Norwegian Institute of Fisheries and Aquaculture Research** Pb 6122 9291 Tromsø Norway
Aspenes	Frode	frodea@nfh.uit.no	Engineer	**Norwegian College of Fishery Science** Varden 14 9018 Tromsø Norway
Birkeland	Nils-Kåre	nils.birkeland@bio.uib.no	Professor	**University of Bergen** Jahnebakken 5, Boks 7800 5020 Bergen Norway
Bjørkmann	Øyvind	oyvind.bjorkmann@nhd.dep.no	Adviser	**Ministry of Trade and Industry** Postboks 8014 Dep 0030 OSLO Norway
Braun	Nick	chefnb@sbc.su.se		**University of Tromsø** Institute of medical biology N-9037 Tromsø Norway
Breivik	Harald	harald.breivik@hydro.com		**Hydro Pronova** Postboks 2560 3942 Porsgrunn Norway
Bruhjell	Stig	stig.bruhjell@student.uib.no	Student	**University of Bergen** Halvkannesmauet 4 5011 Bergen Norway
Burkow	Ivan	ivan.c.burkow@fiskerforskning.no	Director	**Norwegian Institute of Fisheries and Aquaculture Research** Postboks 6144 9291 TROMSØ Norway
Camilio	Ketil Andre	kac022@student.uit.no	Student	**University of Tromsø** Marin bioteknologi, Smørblomstveien 11 9100 Kvaløysletta Norway

Castor	Trevor	tcastor@aphios.com	CEO	**Aphios Woburn** MA 01801 3-E Gill Street Corporation USA
Danson	Michael	bssmjd@bath.ac.uk	Professor	**Centre for Extremophile Research** Department of Biology and Biochemistry, University of Bath BATH BA2 7AY UK
Døskeland	Stein Ove	stein.doskeland@biomed.uib.no	Professor	**University of Bergen** BBB, Jonas Lies vei 91 5009 Bergen Norway
Eggset	Guri	guri.eggset@norinnova.no	Program Coordinator	**MABIT/NorInnova AS** Pb 6413 - Forskningsparken 9294 Tromsø Norway
Eijsink	Vincent	Vincent.eijsink@ikbm.nlh.no	Professor	**Agricultural University of Norway** IKBM, P.O. Box 5003 N-1432 Ås Norway
Elde	Morten	morten.elde@biotec.as	Scientist	**Biotec Pharmacon ASA** Strangata 3 9008 Tromsø Norway
Ellingsen	Trond Erling	Trond.E.Ellingsen@sintef.no	Research Director/ Adjunct Professor	**SINTEF** SINTEF Materialer og Kjemi 7465 Trondheim Norway
Elvevoll	Edel	edele@nfh.uit.no	Professor	**Norwegian College of Fishery Science** Breivika 9037 Tromsø Norway
Enger	Øivind	oivind.enger@forinnova.no	Project Manager	**Forinnova AS/SARSIA Innovation** Thormøhlensgate 55 5008 Bergen Norway
Esaiassen	Margrethe	margrethee@nfh.uit.no		**Norwegian College of Fishery Science** University of Tromsø 9037 Tromsø Norway
Espelid	Sigrun	sigrune@fagmed.uit.no	Scientist	**University of Tromsø** Avd. for molekylær bioteknologi, IMB, UiT 9037 Tromsø Norway
Fenton	Chris	chrisf@fagmed.uit.no	Research Fellow	**University of Tromsø** Thor Knutsensvg 6 9006 Tromsø Norway
Gabrielsen	Kjersti Lie	kjersti.lie.gabrielsen@adm.uit.no	Project Manager Marbank	**University of Tromsø/Marbank** Øvre Lysthus 9037 Tromsø Norway
Gildberg	Asbjørn	asbjorn.gildberg@fiskeriforskning.no	Scientist	**Norwegian Institute of Fisheries and Aquaculture Research** POB 6122 9291 Tromsø Norway

Gjellesvik	Dag Rune	dag.rune.gjellesvik@biotec.as	Research Director	**Biotec Pharmacon ASA** Strandgt. 3 N-9008 Tromsø Norway
Goksøyr	Anders	anders@biosense.com	R&D Director	**Biosense Laboratories AS** Thormøhlensgate 55 5008 Bergen Norway
Gutierrez	Miguel	miguel.angel.gutierrez@tine.no	Senior Researcher	**Tine BA** Søndreskogveien 76 5055 Bergen Norway
Hansen	Espen	espenh@nfh.uit.no	Scientist	**University of Tromsø** IB/Mat.Nat 9037 Tromsø Norway
Hansen	Geir Åsmund	gah022@student.uit.no	Student	**University of Tromsø** IMAB, marin bioteknologi, Conrad Holmboes vei 14 9011 Tromsø Norway
Haug	Tor	torh@nfh.uit.no	Associate Professor	**Norwegian College of Fishery Science** IMAB 9037 Tromsø Norway
Hayes	Ben	ben.hayes@akvaforsk.nlh.no	Senior Researcher	**AKVAFORSK** P.O. Box 5010 N-1432 Ås Norway
Helstad	Kjetil	kjetil.helstad@tromsfylke.no	Adviser	**Troms Fylkeskommune** Pb. 6600 9296 Tromsø Norway
Hjerde	Erik	erikh@fagmed.uit.no	PhD Student	**University of Tromsø** IMB Senjaveien 62a 9012 Tromsø Norway
Hough	Edward	Edward.Hough@chem.uit.no	Professor	**University of Tromsø/NORSTRUCT** Inst. of Chemistry 9037 Tromsø
Jakobsen	Jonas	jonas.jakobsen@fagmed.uit.no	Laboratoy-assistant	**University of Tromsø** AMB, IMB, MH-bygget 9037 Tromsø Norway
Jakola	Karl-Johan	karl.j.jakola@norinnova.no	Director	**NorInnova AS** Boks 6413, Forskningsparken 9492 Tromsø Norway
Jessen	Emil B.	emil.b.jessen@invanor.no	Senior Adviser	**Innovation Norway** Pb 448, Sentrum 0104 OSLO Norway
Jørgensen	Trond	trondj@nfh.uit.no	Professor	**Norwegian College of Fishery Science** Breivika 9037 Tromsø Norway

Jøstensen	Jens-Petter	jens.petter.jostensen@eximo.no	R&D Manager	**Eximo AS** Roald Amundsenspl.1 9253 Tromsø Norway
Kanounova	Olga	ol_og_an@hotmail.com	Student	**Norwegian College of Fishery Science** Stakkevollvn 168F 9010 Tromsø Norway
Kanstad	Marianne Bothner	marianne@stellapolaris.no	Scientist	**Stella Polaris AS** Kårvikhamn 9300 Finnsnes Norway
Kantola	Kristina Garfjell	kristina.kantola@norinnova.no	Trainee	**NorInnova AS** Beringstredet 15 9010 Tromsø Norway
Karlsen	Solveig	solveig.karlsen@chem.uit.no	Senior Engineer	**University of Tromsø** Department of chemistry, Faculty of Science 9037 Tromsø Norway
Kolsvik	Elin	elkol@invanor.no	Adviser	**Innovation Norway** avd. Troms Pb 6304 9293 Tromsø Norway
Kükürtçü	Bulent	bkukurtcu@pharmamar.com	Biodiversity / R&D	**PharmaMar SA** Avda. de Los Reyes, 1 PI La Mina Norte, Colmenar Viejo / MADRID 28770 SPAIN
Landfald	Bjarne	bjarnel@nfh.uit.no	Associate Professor	**Norwegian College of Fishery Science** University of Tromsø 9037 Tromsø Norway
Langgård	Bjørn	bjorn.langgard@invanor.no	Industry Adviser	**Innovation Norway** 133 Federal Street Suite 901 Boston, MA 02110, USA
Larsen	Atle	atlel@fagmed.uit.no		**University of Tromsø** Department of Molecular Biotechnology IMB 9037 Tromsø Norway
Larsen	Per-Ivar	per.ivar.larsen@spindaj.no	Consultant	**Spindaj** Spildra 9185 Spildra Norway
Lian	Stein	nord@onsagers.no	Senior Engineer	**Onsagers Nord** Søndre Tollbodgt. 3a 9008 Tromsø Norway
Lorentzen	Marit Sjo	maritl@fagmed.uit.no	Research Fellow	**University of Tromsø** AMB/IMB 9037 Tromsø Norway
Lynum Pedersen	Hege	hegel@fagmed.uit.no	Research Fellow	**University of Tromsø** MH-bygget, IMB/AMB 9037 Tromsø Norway

Lødemel	Jørgen Blikra	jbl@probio.no	R&D Manager	**ProBio R&D** Pb 6409 Forskningsparken 9294 Tromsø Norway
Manseth	Even	even.manseth@tine.no	Scientist	**Tine BA** Bedriftsveien 7 0902 Oslo Norway
Marvik	Ole Jørgen	o.j.marvik@4bio.no	Partner	**4BIO AS** Kjelsåsveien 174 0884 Oslo Norway
Matland	Anette	Anette.Matland@bio.uib.no	Master Student	**University of Bergen** Institutt for biologi Pb. 139 Rådal 5857 Bergen Norway
McKenzie	Douglas	dmck@integrin.co.uk	CEO	**Integrin Advanced Biosystems Ltd** Marine Resource Centre Barcaldine, Argyll PA37 1SE Scotland
Modalsli	Kristin	kristin.modalsli@forskningsradet.no	Adviser	**The Research Council of Norway** Stensberggt 26 0131 Oslo Norway
Moe	Elin	elinm@fagmed.uit.no	Post. Doc.	**University of Tromsø/NORSTRUCT** 9037 Tromsø Norway
Myrnes	Bjørnar	bjornar.myrnes@fiskeriforskning.no	Senior Scientist	**Norwegian Institute of Fisheries and Aquaculture Research** Muninbakken 9-13 Postboks 6122 N-9291 Tromsø Norway
Måge	Amund	a.maage@online.no	Scientist	**NIFES** Måge 5776 Nå Norway
Niiranen	Laila	lailan@fagmed.uit.no	Research Fellow	**University of Tromsø** IMB, MH-bygget 9037 Tromsø Norway
Nilsen	Gerd	gn@biotec.no	Product Manager	**Biotec Pharmacon** Strandgata 3 9008 Tromsø Norway
Nilsen	Inge W.	inge-w.nilsen@fiskeriforskning.no		**Norwegian Institute of Fisheries and Aquaculture Research** Postboks 6122 9291 Tromsø Norway
Nordrum	Sigve	sigve.nordrum@fid.dep.no	Senior Adviser	**Ministry of Fisheries and Coastal Affairs** Postboks 8118 0032 Oslo Norway
Oftedal	Linn	linn.oftedal@student.uib.no	Research Assistant	**University of Bergen** Trostevn 8 5097 Bergen Norway

Olafsen	Jan A.	janao@nfh.uit.no	Professor	**Norwegian College of Fishery Science** Norwegian College of Fishery Science, UiT N-9037 Tromsø Norway
Olsen	Ragnar	ragnaro@nfh.uit.no	Professor	**Norwegian College of Fishery Science** Breivika 9037 Tromsø Norway
Olufsen	Magne	magne.olufsen@chem.uit.no	Ph.D Student	**University of Tromsø/NORSTRUCT** Vinkelveien 7 9009 Tromsø Norway
Os	Vibeke	vibekeo@chem.uit.no	Senior Engineer	**University of Tromsø/NORSTRUCT** Faculty of Science 9037 Tromsø Norway
Paulsen	Steinar	steinarp@fagmed.uit.no	Post.Doc.	**University of Tromsø** IBM, MH-bygget 9037 Tromsø Norway
pedersen	Alice Marie	alice.marie@tiscali.no	Master Student	**Norwegian College of Fishery Science** Aldor Ingebrigtsensv 27 9010 Tromsø Norway
Pedersen	Zølvi	zolvi.pedersen@invanor.no	Project Manager	**Innovation Norway/Bioklynge Nord** Postboks 6304 9293 Tromsø Norway
Præbel	Kim	kimp@nfh.uit.no	Research Fellow	**Norwegian College of Fishery Science** Breivika 9037 Tromsø Norway
Rasmussen	Tone	tone@sea-eco.com	Manager	**SEA ECO AS/ Biopolymer AB** Havnepromenaden 11 9405 Harstad Norway
Remme	Jannicke Fugledal	jfr@mfaa.no	Scientist	**Møreforsking Ålesund** P.O. box 5075 6021 Ålesund Norway
Robinson	Nick	nick.robinson@akvaforsk.nlh.no	Leader Genetics and Breeding	**AKVAFORSK** PO box 5010 1432 Ås Norway
Ræder	Inger Lin Uttakleiv	ingerlin@chem.uit.no	Ph.D Student	**University of Tromsø** Department of chemistry Breivika 9037 Tromsø Norway
Raa	Jan	jan.raa@biotec.as	Research Director	**Biotec Pharmacon ASA** Arbinsgt. 4 0253 Oslo Norway
Salvesen	Ingrid	ingrid.salvesen@sintef.no	Senior Research Scientist	**SINTEF Fisheries and Aquaculture** Rotvoll Research Centre N-7465 Trondheim Norway

Sandaker	Elin	elin.sandaker@fiskeriforskning.no	Research Technician	**Norwegian Institute of Fisheries and Aquaculture Research** Muninbakken 9-13 9291 Tromsø Norway
Sandsdalen	Erling	erling.sandsdalen@fiskeriforskning.no	Research Director	**Norwegian Institute of Fisheries and Aquaculture Research** Postboks 6122 9291 TROMSØ Norway
Seliussen	Bjørghild	bjobre@student.uit.no		**University of Tromsø** Olastien 15, 202 9012 Tromsø Norway
Skjærven	Kaja	Kaja.skjarven@iac.uib.no	Research Assistant	**University of Bergen** Tartargt. 16 5034 Bergen Norway
Smalås	Arne O.	Arne.Smalas@chem.uit.no	Professor	**University of Tromsø/NORSTRUCT** 9037 Tromsø Norway
Solem	Stein Tore	steins@nfh.uit.no	Scientist	**Norwegian College of Fishery Science** Institutt for Marin Bioteknologi Breivika 9037 Tromsø Norway
Stangeland	Janne	janneks@student.uit.no	Student	**University of Tromsø** blåklokkevn.46 9100 Kvaløysletta Norway
Stenberg	Even	even.stenberg@fiskeriforskning.no		**Norwegian Institute of Fisheries and Aquaculture Research** Breivika 9291 Tromsø Norway
Stensvåg	Klara	klaras@nfh.uit.no	Associate Professor	**Norwegian College of Fishery Science** Breivika 9037 Tromsø Norway
Strand	Mette	mette@holmfjord.no		**Holmfjord AS** Holmfjord 9716 Børselv Norway
Styrvold	Olaf B.	olafs@nfh.uit.no	Associate Professor	**Norwegian College of Fishery Science** Breivika 9037 Tromsø Norway
Støen	Mariel Aguilar	mariel.stoen@sum.uio.no	Research Fellow	**University of Oslo/SUM** Po box 1116 Blindern NO-0317 Oslo Norway
Størseth	Trond R.	trond.r.storseth@sintef.no	Research fellow	**SINTEF Fisheries and Aquaculture** Arkitekt Ebbells vei 10 7465 Trondheim Norway
Søreide	Fredrik	fredrik.soreide@vm.ntnu.no		**NTNU** Skovvn 39 B 0258 Oslo Norway

Tande	Kurt	www.calanus.no	Manager	**Calanus AS** P.O. Box 2489 9272 Tromsø Norway
Thomson	Nicholas	nrt@sanger.ac.uk		**The Wellcome Trust Sanger Institute** Wellcome Trust Genome Campus, Hinxton Campus, CB10 1SA UK
Van Belle	Werner	werner.van.belle@itek.norut.no	Researcher	**Norut Itek** Malmveien 206 9022 Krokelvdalen Norway
Viswanath	Kiron	kiron.viswanath@hibo.no	Professor	**Bodø University College** Dept. of Fisheries & Natural Sciences 8049 Bodø Norway
Vögele	Barbara	bv@akvaplan.niva.no	Consultant	**Akvaplan-niva** Polarmiljøsenteret 9296 Tromsø Norway
Vårum	Kjell M.	kvaarum@biotech.ntnu.no	Professor	**NTNU/NOBIPOL** Dep. of Biotechnology Sem. Sælands v. 6/8 7491 TRONDHEIM Norway
Wang	Ellen	ellen.wang@chem.uit.no	Research Fellow	**University of Tromsø** Rederveien 58 9014 Tromsø Norway
Willassen	Nils Peder	nilspw@fagmed.uit.no	Professor	**University of Tromsø/NORSTRUCT** Inst. of mol. biotec., 9037 Tromsø Norway
Wu	Peilan	wu.peilan@invanor.no	Market Advisor	**Innovation Norway, Shanghai Office** Royal Norwegian Consulate General, Shanghai. 306 Zhong Shan Dong Yi Road, Shanghai 200002 P.R.China
Yang	Nannan	nannan@chem.uit.no	Post.Doc.	**University of Tromsø/NORSTRUCT** Det matematisk- naturvitenskapelige fakultet 9037 Tromsø Norway
Zotchev	Sergey	sergey.zotchev@biotech.ntnu.no	Associate Professor	**NTNU** Institutt for bioteknologi S. Sælands v 6/8 7491 Trondheim Norway
Øverbø	Kersti	kersti.overbo@fiskeriforskning	Engineer	**Norwegian Institute of Fisheries and Aquaculture Research** Muninbakken 9291 Tromsø Norway